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Summer heat and an epidemic of cytospora canker of *Alnus*

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Abstract: An epidemic of cytospora canker [*Valsa melanodiscus*, anamorph *Cytospora umbrina*] is associated with dieback and mortality of *Alnus incana* ssp. *tenuifolia* in the Southern Rocky Mountains and had begun by the late 1980s. Isolations showed that inoculum was often abundant on bark and bud surfaces even in winter, especially near diseased stems. The pathogen was occasionally isolated from internal tissues of dormant buds and asymptomatic wood and phloem. In infected stems, the pathogen was detected up to 5 cm beyond visible canker margins. The data suggest that the pathogen can cause latent infections, but the infection court remains unclear. Inoculations of healthy stems in the field did not induce canker formation. Fruiting of the anamorph was primarily in late winter and spring, and sexual maturation occurred in late summer and fall. Stem age and growth data support earlier conclusions that the mortality does not represent a steady-state condition with normal attrition of older stems. Canker expansion and killing of branches and stems occurred almost exclusively in the warmest part of summer. We present a hypothesis and supporting evidence suggesting that warm summer temperatures are conducive to the disease. Locally, summer temperatures, and especially maxima, have varied with a dominant oscillation period of approximately 21 years. We speculate that alder populations expand during cool climate phases and shrink during warm phases due to epidemics of cytospora canker. More recently, the oscillation has dampened as an increasing trend of temperature has become dominant, locally and globally. If the trend continues, this already severe epidemic may become more damaging, without intervening opportunities for alder populations to recover.

Keywords: *Alnus incana* ssp. *tenuifolia*, climate change, cytospora canker, endophyte, latent infection, mortality, temperature oscillation, thinleaf alder, *Valsa melanodiscus*, water potential

Résumé: Une épidémie de chancre cytosporéen (*Valsa melanodiscus*, anamorphe *Cytospora umbrina*), qui a débuté vers la fin des années 80, est associée au dépérissement et à la mortalité chez *Alnus incana* ssp. *tenuifolia* dans le sud des montagnes Rocheuses. Les isolements ont montré que l'inoculum abondait sur l'écorce et les bourgeons, même en hiver, près des tiges infectées. L'agent pathogène a parfois été isolé à partir de tissus internes des bourgeons dormants ainsi que de bois et de phloème asymptomatiques. Dans les tiges infectées, l'agent pathogène a été détecté jusqu'à 5 cm au-delà des bords visibles du chancre. Les données suggèrent que l'agent pathogène peut causer des infections latentes, mais que le point d'entrée infectieux demeure incertain. Des inoculations de tiges saines sur le terrain n'ont pas provoqué la formation de chancre. La fructification de l'anamorphe avait essentiellement lieu à la fin de l'hiver et au printemps, tandis que la maturation sexuelle se produisait à la fin de l'été et à l'automne. L'âge des tiges et les données sur la croissance appuient les conclusions précédentes selon lesquelles la mortalité ne représente pas un état continu quant à la diminution du nombre des plus vieilles tiges. L'accroissement du chancre de même que la mort des branches et des tiges se produisaient presque exclusivement durant la période la plus chaude de l'été. Nous présentons une hypothèse, avec preuves à l'appui, qui suggère que les températures chaudes de l'été contribuent à la progression de la maladie. Localement, les températures estivales, et particulièrement les maximums, ont varié en fonction d'une période dominante d'oscillation d'environ 21 ans. Nous présumons que les populations d'aulne s'accroissent durant les phases de climat frais et décroissent durant les phases chaudes à cause des épidémies de chancre cytosporéen. Récemment, l'oscillation s'est stabilisée étant donné qu'une tendance au réchauffement, local et mondial, prévaut. Si la tendance se maintient, cette épidémie qui est déjà grave pourrait s'aggraver encore davantage, ne laissant aucune chance aux populations d'aulnes de récupérer.

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Mots clés: *Alnus incana* spp. *tenuifolia*, aulne à feuilles minces, champignon endophyte, chancre cytosporéen, changement climatique, infection latente, mortalité, oscillation des températures, potentiel hydrique, *Valsa melanodiscus*

Introduction

High incidence of branch dieback and mortality of thinleaf alder, *Alnus incana* (L.) Moench ssp. *tenuifolia* (Nuttall) Breitung, has been noted in western North America in recent years. Substantial damage, with 37% of standing stems dead and 29% diseased, was documented in the Southern Rocky Mountains, from northern New Mexico, through Colorado, and into southern Wyoming (Worrall, 2009). Very similar damage has been observed in south-central and interior Alaska (Trummer, 2006; Ruess *et al.*, 2009), and has been noted also in British Columbia (L. Trummer, personal communication). Cytospora canker, caused by the fungus *Valsa melanodiscus* G.H. Otth (anamorph *Cytospora umbrina* (Bonord.) Sacc.), is strongly associated with and is the proximate cause of the damage (Trummer, 2006; Stanosz *et al.*, 2008; Worrall, 2009).

Documentation of this widespread epidemic, or epidemics, raises several important questions. Why has this apparently native, stress-related pathogen become so prevalent and damaging over such a wide area? The epidemic in Alaska apparently began in 2002–2003 (Trummer, 2004, 2006), while in Colorado it apparently began by the late 1980s (Worrall, 2009), so the answers to this question may differ between these areas. How and where does the pathogen infect host tissue? Although some pathogens that cause cytospora cankers may infect through wounds, others may cause latent infections, establishing in healthy tissues and causing disease following some stimulus (Adams *et al.*, 2005).

The objectives of this study were to determine: (a) where and how frequently the pathogen occurs on plant surfaces and inside living tissues of thinleaf alder in the Southern Rocky Mountains; (b) whether the pathogen is virulent in field inoculations; (c) when fruiting of the anamorph and teleomorph occur; and (d) the phenology of canker growth and related implications regarding climatic drivers of epidemics.

Materials and methods

Study sites

Studies were conducted in thinleaf alder stands throughout the upper Gunnison River basin in Colorado, USA, located in the Southern Rocky Mountain physiographic province (Fenneman, 1946) within the Southern Rocky

Mountain Steppe–Open Woodland–Coniferous Forest–Alpine Meadow ecoregion province (Bailey, 1995). In this basin, alder occurs along streams between about 2350 to 3050 m elevation. In some stands, alder is the tallest plant, but it also occurs frequently in association with *Populus tremuloides* Michx., *P. angustifolia* James, *Picea engelmannii* Parry ex Engelm., and *P. pungens* Engelm.

Isolations

Isolations from plants without cankers were conducted monthly from February to August to gain information about occurrence of inoculum and possible latent colonization. On each sample date, 9–12 stems were sampled and 8–12 pieces were plated from each sample and tissue type. Surface isolations were made by directly plating small pieces of untreated surface tissues (such as whole winter buds or 3 mm square, tangential slices of outer bark) onto acidified malt-extract agar (AMA) (Worrall, 2009). Other isolations were conducted after surface sterilization. Buds (available February through May only) were surface-sterilized by soaking for 1 min in a solution containing 10% household bleach (final concentration of NaClO approximately 0.5%), 10% ethanol and 80% water, then rinsing in sterile water. Shoots, ranging from first-year growth to wedges split from large stems, were cut into 4 cm segments and surface-sterilized in 95% ethanol for 1 min, 50% bleach for 5 min, 95% ethanol for 30 seconds, followed by briefly flaming off the residual ethanol. After surface-sterilization, buds were aseptically sliced lengthwise and internal parts plated. Isolations from shoots were taken from at least 1 cm away from the cut ends after surface-sterilization. Bark squares were separated and plated cambium-side down. Wood samples were taken from the outer 10 mm.

Isolations were also made systematically around cankers to determine if the pathogen was present outside the visible canker margin, and if so, in what tissues. Eight stems with cankers were collected during October and November, 2006. Stems were surface-sterilized by swabbing with bleach–ethanol solution (see above). Starting at the margin of the canker, 3 mm squares were removed aseptically from the phloem and underlying wood (to c. 2 mm deep) at 1 cm intervals out to 10 cm and plated on AMA. Such isolation strips were done at each side, the bottom, and the top of the canker when it was accessible.

Inoculations

Two sets of field inoculations were conducted using cultures freshly isolated from cankers. Genets were used as blocks, with each treatment on a different stem of the genet. Stems approximately 2.5–4.0 cm diameter were chosen. The site was swabbed with 95% ethanol, a wound created (in the first experiment this was a stab with a sterile needle; in the second, bark was removed with a sterile, 4 mm cork borer), and a 4 mm disk from nonsporulating cultures on malt-extract agar was placed over the wound. The site was wrapped with parafilm and in the first experiment also with aluminium foil. In the first experiment, performed 25 September 2003, only one isolate was used and treatments were (a) wounded inoculation as described above; (b) inoculation without the wound; and (c) a wounded control with no fungus. In the second experiment, performed 2 August 2006, treatments were three different isolates used in wounded inoculations and an uninoculated, wounded control. In both experiments, 10 replicate genets were used.

Fruiting phenology

Occurrence of conidiomata and ascomata in naturally occurring cankers on live stems was monitored from April through November, 2006. Thin horizontal and vertical sections of stromata were cut under a dissecting microscope and mounted in 3% KOH for viewing in a compound microscope. The anamorph and teleomorph of *Valsa melanodiscus* were identified based on published descriptions (Spielman, 1985).

Age, growth and disease

To determine the ages of alder stems and the relationship between age, recent changes in growth rate, and the disease, we sampled a total of 129 alder stems from 26 sites in June and July, 2005. We selected a broad range of stem diameters for sampling, from the largest found on each site down to 3 cm, and also selected stems with a range of disease symptoms from healthy to recently killed. For every stem, we estimated percentage crown dieback and cut a disc from the base. After drying, the discs were sanded, rings were counted, and the width of each five-year increment was estimated by averaging measurements made with a micrometre along the longest and shortest radius.

Canker phenology

Growth of 50 stem cankers was monitored from March through October, 2006 in 19 locations throughout the upper

Gunnison River basin, Colorado. A nail and tag were placed in the centre of each canker and the stem circumference measured at the nail. Canker margins were marked with a paint pen at the sides, top and bottom and measured from the nail. Cankers were remeasured monthly. When a second canker on the stem merged with the first, subsequent measurements encompassed the combined canker. In many cases, the tops became too high to measure, often girdling at some point above the nail and killing the entire upper stem. When the stem was girdled at the height of the nail, the cankers were no longer revisited. Canker size was expressed as percentage of the circumference that was killed (per cent girdling).

Weather and climate

We examined the weather records for station Gunnison 3SW (Coop ID 053662) for the period during which we monitored canker growth, March through October, 2006. The station is centrally located, ranging from 13 to 28 km from the monitored cankers.

Long-term climate data for station 3SW were obtained from the National Oceanic and Atmospheric Administration's National Climatic Data Center (<http://cdo.ncdc.noaa.gov/CDO/cdo>). Data were subjected to spectral analysis as follows. First, a summer heat index (SHI) was calculated using the general methodology of and the same reference period as Hansen *et al.* (1998, further described at <http://data.giss.nasa.gov/csci/>). The three variables used were monthly extreme maximum temperature, the monthly mean maximum temperature, and the monthly mean temperature. For each year, each variable was averaged over the months of June, July and August. The mean of the reference period (1951–1980) was subtracted from the value for each year and the result divided by the standard deviation of the mean for the reference period. The resulting values for the three variables were averaged to obtain the annual SHI value. Various periodograms and evolutive spectra were used to assess the periodicity of the data using the SSA-MTM Toolkit for Spectral Analysis (Ghil *et al.*, 2002).

Results

Isolations

Of 2236 tissue pieces plated from healthy stems, *V. melanodiscus* was isolated from 184 (8.2%). Most isolations were from exposed surfaces: 21% of buds and 24% of bark surface pieces (Table 1). Observations suggested that frequent isolation from bark occurred when collections were made near infected stems, but we did not test this

Table 1. Isolations of *Valsa melanodiscus* from various alder tissues.

Tissue/organ		Number of pieces	Percentage with <i>V. melanodiscus</i>
Internal	phloem	657	0.3
	xylem	699	1.6
	buds	187	4.8
Surface	buds	104	21.2
	bark	589	23.8
TOTAL		2236	8.2

hypothesis. Internally, the most frequent isolation was from healthy buds (4.8%). The pathogen was isolated less commonly from healthy xylem (1.6%) and phloem (0.3%). Isolation frequency varied greatly among sampled stems and no trend of isolation frequency with time was observed.

Around cankers, the pathogen was isolated from xylem and/or phloem as far as 5 cm from the visible canker margin (Fig. 1). *Valsa melanodiscus* was not isolated from five of the eight cankers, all of which had abundant contaminants. Another canker yielded *V. melanodiscus* 1–5 cm below and 4–5 cm to one side of the canker. The results from the remaining two cankers are diagrammed in Fig. 1.

Inoculations

Inoculations resulted in no canker formation or other evidence of infection during post-inoculation observations of 2.5 yr (first experiment) or 1 yr (second experiment). Inoculated treatments were indistinguishable from controls. In both, healthy callus growth at the wound margin was consistently observed. In one case, a naturally occurring canker had grown from below over the inoculation site when examined after 1 yr.

Fruiting phenology

In April, only the anamorph was found. At this time of year, in two successive years, we have frequently observed well-developed cirrhi on conidiomata in the field. No samples were collected in May. In June and early July, mature ascomata were found. In late July and August, only immature ascomata were found. In September through November, mature ascomata were again found.

Age, growth and disease

Sampled stems ranged from 8 to 66 years old. Severity of crown dieback did not vary significantly with stem age (Fig. 2a), and mortality (100% dieback) occurred across

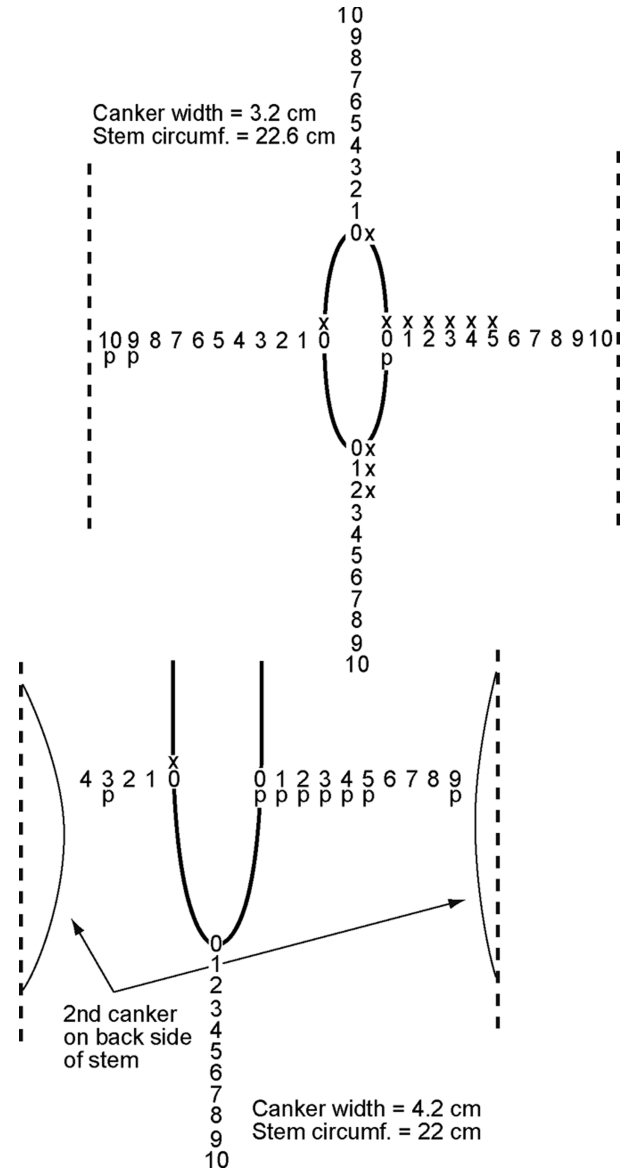


Fig. 1. Isolation of *Valsa melanodiscus* surrounding two cankers on different stems. Dashed lines are where the two sides of each drawing meet on the back side of the stem. Numbers are distances in cm from the canker margin. 'x' represents isolation of *V. melanodiscus* from the xylem at that position; 'p' from the phloem. Other fungi grew from most chips and may have limited isolation of *V. melanodiscus*. Thus, positions from where *V. melanodiscus* was not isolated should not be interpreted as positions at which it is absent.

the range of ages. Similarly, no significant relationship was found between amount of dieback and changes in radial growth rate between the last 10 years and the previous 10 years (Fig. 2b). Other tested growth ratios, such as the corresponding five-year periods, showed even less association with disease. Mortality occurred even in

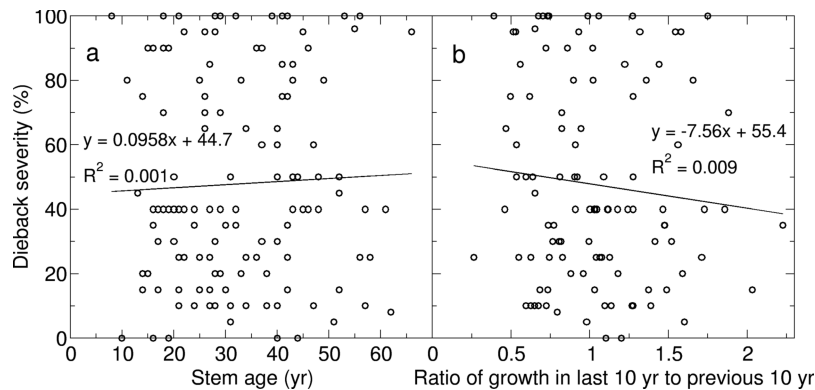


Fig. 2. Lack of relationship between severity of crown dieback versus (a) stem age, and (b) recent growth changes. Number of stems included is 129 for (a) and 106 for (b). Fewer stems were available for (b) because only stems ≥ 20 years old could be used. Crown dieback was estimated in increments of 5%.

stems whose growth in the last decade was 1.5–2.0 times greater than in the previous decade. Thus, dieback was independent of age, and stems did not decrease growth significantly between the two five-year or 10-year periods before dieback was measured.

Canker phenology

Of 50 cankers marked for measurement in 2006, 10 grew less than 5 points in per cent girdling (nine did not grow at all). One additional canker was dropped because the stem was cut by beavers during the summer. Of those that grew significantly and were monitored throughout the period, most grew little or not at all up to mid-June, but grew extensively between the 20 June and 20 July measurements (Fig. 3a).

The average growth (heavy dashed line in Fig. 3a) shows some increase from mid-April to mid-June before the large increase, but this is due to substantial growth of only a few anomalous cankers. The cankers that grew most in the early summer were on ramets whose tops had already been largely killed by the pathogen the previous year. Only five cankers girdled their stems between March and mid-June, but then almost all cankers grew rapidly and 22 girdled in the interval before the next measurement in mid-July. Most did not grow at all after mid-July.

Vertically, cankers grew as fast as 50 cm per month in one direction. However, growth rate was highly variable.

Weather and climate

In the period during which we monitored canker growth, the 10-day moving average of daily mean and maximum temperatures generally rose steeply until mid-June, when there was a brief dip before rising again (Fig. 3b).

Between the 20 June and 20 July measurements, which encompassed the extensive canker growth, maximum temperature was ≥ 25 °C on 24 days, ≥ 27 °C on 18 days, and peaked at ≥ 30 °C on four consecutive days. The warm period was then broken by two days with the highest precipitation of the summer. This marked the arrival of the summer monsoon climate pattern. Precipitation occurred on 21 days of the first four weeks of the monsoon, while temperature dropped to daily means generally below 15 °C and daily maxima mostly below 25 °C.

Long-term climate data were analyzed to determine if climate patterns could account for the long-term epidemic of cytospora canker in alder. Positive values of the summer heat index (SHI) indicate temperatures warmer than the mean of the reference period (1951–1980) and vice versa (Fig. 4e). Blackman–Tukey and maximum entropy spectra both showed a strong peak with a frequency of 0.047 cycles yr^{-1} , or a period of 21.3 yr (Figs. 4a and 4b). Multi-taper analysis led to rejection of the red-noise null hypothesis for the peak ($P < 0.01$; Fig. 4c). Singular spectrum analysis showed the signal as two components forming an oscillating pair above the 95% confidence interval and widely separated from the rest of the spectrum (Fig. 4d). Reconstruction of that oscillation showed a good correspondence with variation in the index (Fig. 4e).

The oscillation was particularly regular with high amplitude in the middle of the 20th century, with alternating cool and warm phases lasting about a decade each. However, the oscillation gradually became slower and amplitude dampened after the late 1970s. The last clear phase shift, in 1976, was to a warm phase, and a subsequent phase shift cannot be reliably identified. Previous warm phases of the SHI were interrupted by phases with much cooler summers. In the last SHI cool phase, 1963–1976, only 1 of 42 summer months (2.4%) recorded

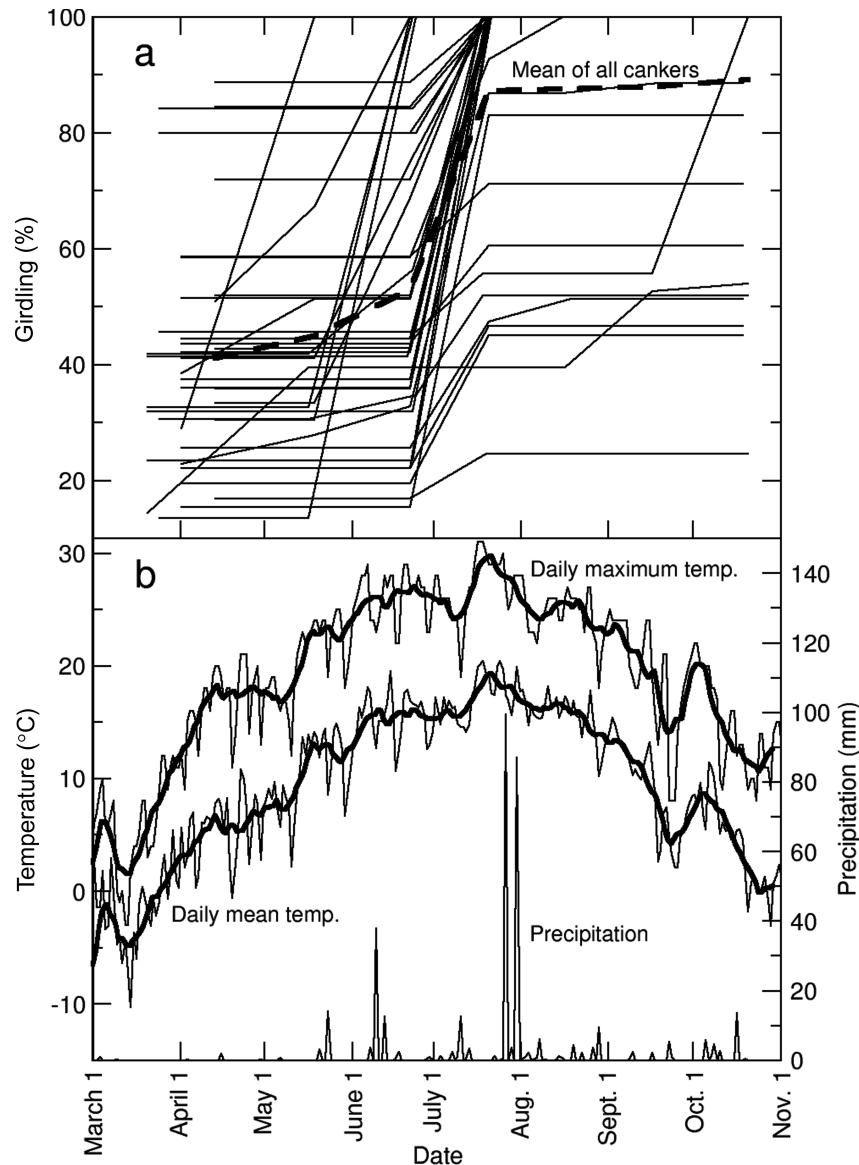


Fig. 3. **a**, Development of 39 marked cankers that grew significantly during March–October 2006, expressed as per cent girdling (complete growth of canker around the circumference). The bold dashed line is the average of all cankers. **b**, Weather at the Gunnison 3sw weather station for the corresponding period. Heavy lines are 10-day moving averages of temperatures.

extreme maximum temperatures above 32 °C. Since 1976, the SHI has not been exceptionally high, but there are many peaks of a height similar to previous warm phases. Ten of the 80 summer months (12.5%) since 1976 had temperatures above 32 °C. Moreover, since 1976 there have been no significant intervening cool periods comparable to earlier ones.

Discussion

Our isolations indicate that inoculum of *Valsa melanodiscus* is abundant on bark and bud surfaces. Inside healthy

tissues, *V. melanodiscus* occurred most frequently in winter buds, and to a lesser extent in xylem and phloem. In buds, it is possible that spores may have become embedded in the resin coating and thus been protected from surface sterilization, but this would not account for isolations from xylem and phloem. Internal isolations in the absence of cankers represent latent infections. Isolation of the pathogen from xylem and phloem up to 5 cm from visible canker margins indicates that the pathogen can grow in live tissues without causing immediate symptoms, and lends support to the likelihood of latent infections.

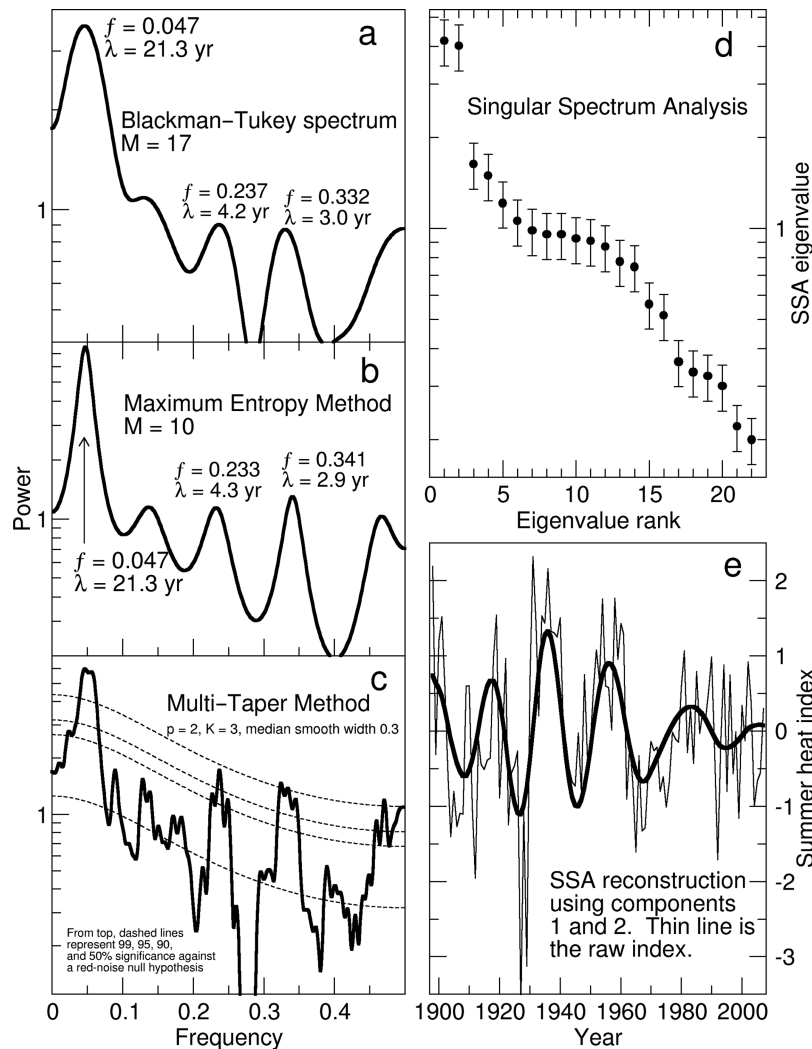


Fig. 4. Spectral analysis of Gunnison summer heat index from 1898–2007 using **a**, Blackman–Tukey, **b**, Maximum Entropy Method, **c**, Multi-Taper Method, and **d**, Singular Spectrum Analysis. The first pair of components in **(d)** represent a significant oscillation ($f = 0.047$) corresponding with significant oscillations in the other methods and which is reconstructed in **(e)** against the raw index.

The initiation of cankers in the absence of wounding or twig stubs (Worrall, 2009) could also be explained if the pathogen became established early in tissue development.

Certain *Cytospora* species can cause latent infections, colonizing living tissues asymptotically, presumably later inducing symptoms when conditions become conducive. *Picea pungens* inoculated with *C. kunzei* was colonized regardless of stress, but only stressed plants exhibited canker symptoms, and the fungus was also present several centimetres beyond visible symptoms (Schoeneweiss, 1983). Although not common, *Cytospora* species have been isolated from bark and xylem of asymptomatic hosts in a number of species (Chapela, 1989; Adams *et al.*, 2005).

Although our inoculations did not result in canker formation, two other studies of this pathosystem in Colorado and Alaska did observe canker development following inoculation (Kepley & Jacobi, 2000; Stanosz *et al.*, 2008). Because stress and physiological condition of the host are important influences on susceptibility to *Cytospora* cankers, it is not unusual to experience difficulty in inducing disease (Bier, 1964; Adams *et al.*, 2005). In greenhouse inoculations, stress must often be induced for inoculations to cause disease (Bloomberg, 1962; Schoeneweiss, 1975; Guyon *et al.*, 1996; Kepley & Jacobi, 2000). Our field inoculations (2 August and 25 September) may have missed the periods of moisture stress when resistance is compromised and the pathogen can readily develop in host tissues (see below).

In the Southern Rocky Mountains, our observations have consistently indicated presence and heavy sporulation of conidiomata in late winter and early spring. We have not sampled during late autumn and most of the winter, but the anamorph may be present during that time as well. Ascomata that we found in June and early July were mature and sporulating, but in late July and August we found only immature ascomata. Mature ascomata were again found consistently in September through November. Ascomata apparently develop during summer and mature in autumn; we suspect that the mature ascomata found in early summer had overwintered and resumed sporulation.

These observations are limited, and may not apply in other areas. In south-central to interior Alaska we (with Lori Trummer) found both conidiomata and ascomata commonly in late June (unpublished data). Cooler and moister summers there may support continued sporulation of conidiomata as well as overwintered ascomata into the summer.

If the extensive dieback and mortality of alder were a steady-state condition and represented normal attrition of older stems, dieback and mortality of stems above 3 cm DBH (above the size of small sprouts and stems where attrition is high) should increase with stem age. However, we found that dieback and mortality did not increase significantly with age of stems. This supports the conclusion that this damage does not represent steady-state condition of alder and instead represents altered population dynamics. Further support for this conclusion is the failure of genets to successfully replace themselves vegetatively, a stage-based matrix model analysis indicating that a steady state was only remotely feasible, and historical observations indicating that alder condition worsened and caused concern before 1991 (Worrall, 2009).

The lack of decreasing growth rate associated with dieback and preceding mortality suggests that stems generally grew well until shortly before they died, and that any stress that favoured canker growth and dieback was short-lived and acute. This is consistent with the analysis of canker phenology and weather below, suggesting that the stress conducive to canker growth is brief and acute. If host vigour is affected only during the brief period of canker expansion, growth of alder may be relatively unaffected during the rest of the growing season until significant foliage loss or complete mortality occur.

Canker growth was largely restricted to the period from late June to late July, and was rapid and extensive during this period. Exceptions were cankers on trees whose crowns had already largely been killed. Crown loss likely weakened those stems and reduced their ability to resist further canker development earlier in the

summer. The cankers that grew primarily in late June to late July were generally on healthier trees.

The phenology of development of cytospora cankers has rarely been studied, but it appears to be unusual for cankers to grow for a brief period in the middle of the growing season. The most common pattern is active spreading and girdling when trees begin growth in the spring (Adams *et al.*, 2005), probably related to the stress of leaf expansion and the mobilization of nutrients to growing shoot tips. Cytospora canker of *Populus tremuloides* is most active when the host is dormant and incapable of response (Hinds, 1985), although drought stress can lead to canker growth during the growing season (Guyon *et al.*, 1996; McIntyre *et al.*, 1996). Cytospora canker of *Prunus* and cryptodiaportha canker of *Salix* also develop primarily when the hosts are dormant (Bier, 1959; Bertrand *et al.*, 1976). Thus, restriction of canker growth largely to a sharp spike in midsummer appears to be unusual.

Most alders killed in this epidemic were continuously supplied with fresh water around their roots (Worrall, 2009). Yet cytospora cankers are usually stress-related diseases, and moisture stress is the most frequently observed factor involved. The explosion of canker growth associated with high temperatures is consistent with moisture stress, but how could alders suffer moisture stress when they are rooted at the edges of full, active streams?

The physiology of thinleaf alder is little studied, but information is available for other members of the genus, most of which live in habitats where soil moisture is plentiful and consistent. Their response to transpiration-induced water stress during warm, dry atmospheric conditions is of interest. Some woody angiosperms are isohydric (maintaining high xylem Ψ by strict stomatal control) and others anisohydric (allowing xylem Ψ to drop while keeping stomata open) (Tardieu & Simonneau, 1998; McDowell *et al.*, 2008). *Alnus rubra* Bong. and *A. glutinosa* (L.) Gaertn. appear to be anisohydric: they do not respond to decreasing xylem Ψ , but continue to transpire as long as the roots have water (Hacke & Sauter, 1996; Eschenbach & Kappen, 1999). In field measurements, these species maintain maximum stomatal conductance and high transpiration rates when plant Ψ falls to levels at which many species close their stomata (Bond & Kavanagh, 1999; Eschenbach & Kappen, 1999). This has been interpreted as a strategy to maximize productivity on sites where water supply is normally unlimited (Eschenbach & Kappen, 1999).

However, this strategy may impose a cost when warm, dry atmospheric conditions lead to high leaf-to-air vapour pressure gradients and tissue Ψ s at or below levels that

cause cavitation, even with water freely available to the roots (Hacke & Sauter, 1996; Eschenbach & Kappen, 1999). On a day with maximum temperature of only 20 °C, midday leaf Ψ s of *A. glutinosa* fell as low as -1.7 MPa (Eschenbach & Kappen, 1999). On the most stressful day of six that were measured through the season (temperature not reported), the upper leaves had midday Ψ s as low as -2.3 MPa. *Alnus glutinosa* xylem is considered highly vulnerable to cavitation, beginning at about -1.2 MPa, which commonly occurred during midday (Hacke & Sauter, 1996). Similarly, up to 60% loss of hydraulic conductivity due to xylem cavitation occurred in *A. incana* ssp. *tenuifolia* stems at about -1.5 MPa (Hacke *et al.*, 2001). Stomata may respond to soil Ψ , but not to leaf Ψ , so high temperature, low humidity and wind can lead to dangerously low xylem Ψ (Eschenbach & Kappen, 1999; Schrader *et al.*, 2005).

This cost may explain the lower elevational limit of thinleaf alder in the Southern Rocky Mountains, where it occurs generally above 2060 m (Furlow, 1979; Worrall, 2009). At lower elevations, ample moisture is available in the riparian habitat, but alder does not occur. Those lower elevations have higher summer temperatures and lower relative humidity than the mountains. As discussed above, these are conditions for which the water-management strategy of alder is not well suited. Similarly, *Alnus rubra* and *A. glutinosa* are said to avoid rather than tolerate water stress; their strategy does not allow growth in sites that normally subject them to moisture stress (Giordano & Hibbs, 1993; Eschenbach & Kappen, 1999).

Betula occidentalis Hook., a frequently co-occurring riparian species in the interior West, extends to lower elevations than does *A. incana* ssp. *tenuifolia*. For example, *B. occidentalis* grows as low as 1520 m in Colorado and 1210 in Utah (Albee *et al.*, 1988; Uchytel, 1989). An isohydric species, *B. occidentalis* regulates stomatal conductance in response to both soil and leaf water potential (Sperry & Saliendra, 1994; Saliendra *et al.*, 1995). This is consistent with a greater tolerance than *A. incana* ssp. *tenuifolia* of warm, dry atmospheric conditions at low elevations.

Moisture stress commonly predisposes trees to cytospora cankers and other cankers caused by facultative pathogens (Schoeneweiss, 1981; Adams *et al.*, 2005; Sinclair & Lyon, 2005). A variety of woody hosts became susceptible to cankers caused by facultative pathogens when moisture content of bark fell below a threshold of 80% of saturation (Bier, 1959, 1964). Water potentials below -1.2 MPa predisposed a variety of woody plants to such diseases (Schoeneweiss, 1981; Guyon *et al.*, 1996). A strong correlation was found between leaf Ψ and length of cytospora cankers in *Prunus domestica* L.

following inoculation; Ψ s as high as -1.0 MPa were sufficient to induce susceptibility (Bertrand *et al.*, 1976). Similarly, following field inoculations of *Populus tremuloides*, growth of cytospora cankers increased significantly as Ψ decreased (Guyon *et al.*, 1996). In poplar, both high temperature and low tissue moisture content were related to development of cytospora canker (Bloomberg, 1962). Susceptibility may occur under moisture stress too mild to induce significant loss of xylem function. Thus, moisture stress in thinleaf alder is likely to result from warm, dry atmospheric conditions even when roots are amply supplied with water, and this stress, perhaps compounded by xylem cavitation, predisposes the plant to cytospora canker.

The period of explosive canker growth was remarkably constrained to late June to late July, when temperatures were the highest of the summer and humidity is generally low. This is especially true when trees already heavily damaged from canker growth in prior years are excluded from consideration. These observations are consistent with what is known about predisposition to the disease by moisture stress and the physiology of alder, as outlined above.

The period suitable for rapid canker development ended with the arrival of the monsoon pattern. The monsoon of southwestern North America brings southwesterly winds with warm, moist air to the Southern Rocky Mountains (Hales, 1974). Humidity increases and thunderstorms often occur almost daily in the mountains due to adiabatic cooling. This weather pattern typically lasts from mid-July to early September.

Canker growth halted with the arrival of monsoon conditions despite the persistence of high maximum temperatures for several weeks. The increase in atmospheric moisture likely reduced transpiration demand and the associated moisture stress.

On a longer time scale, climate may also explain the onset of the long-term epidemic of cytospora canker, which has been killing thinleaf alder since at least 1990 (Worrall, 2009). The local summer heat index (SHI) has oscillated with high amplitude since records began in 1898, with a period of 21.3 yr (Fig. 4e). If high summer temperatures are conducive for cytospora canker, as the preceding data and discussion suggest, alder likely suffered from epidemics of the disease lasting roughly 10 years during warm phases, with intervening periods of recovery during cool phases. More recently the oscillation has become dampened. Although the index has not been exceptionally high since the late 1970s, there has been no cool, or 'recovery' period, of more than a year or two. The current epidemic may be less intense than some previous ones, but it may also be of longer duration.

Alternatively, or in addition, long-term temperature increase could explain the increase in disease. Initial analyses of the climate over the 20th century in the Southern Rocky Mountains were inconsistent with a hypothesis that long-term temperature trends could explain the epidemic or the condition of alder. Two studies found that temperatures did not increase significantly in the Southern Rocky Mountains during the 20th century (Kittel *et al.*, 2002; Baldwin, 2003 [refers to the region as the East Central Rockies]). However, more recent analyses of annual mean temperatures in Colorado, up to 2006, show evidence of significant upward trends becoming steeper in the last 30 years (Ray *et al.*, 2008). Significant increases between 1977 and 2006 ranged from 0.6 to 1.4 °C in different regions of Colorado. Although the Gunnison summer temperature record does not reflect this trend (Fig. 4e), that of the Cochetopa Creek station (18 km southeast) does (data not shown).

The large amplitude of the oscillation in summer temperature through most of the 20th century suggests that the oscillation likely had a strong influence on the disease, and thus on population dynamics of alder. More recently, a temperature trend appears to have become dominant, while the oscillation has dampened. If our hypothesis relating temperature, water potential and disease is correct, predicted increases in temperature (Core Writing Team *et al.*, 2008) will likely cause the epidemic of cytospora canker to continue and become more severe. This more severe epidemic, with the lack of intervening periods of recovery, would reduce alder populations to a greater extent than earlier epidemics.

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References

ADAMS, G.C., WINGFIELD, M.J., COMMON, R., & ROUX, J. (2005). Phylogenetic relationships and morphology of *Cytospora* species and related telomorphs (Ascomycota, Diaporthales, Valsaceae) from *Eucalyptus*. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. Retrieved from <http://www.cbs.knaw.nl/publications/CBSPublicationDetails.aspx?Rec=1052>.

- ALBEE, B.J., SHULTZ, L.M., & GOODRICH, S. (1988). Atlas of the vascular plants of Utah, Utah Museum of Natural History Occ. Publ. no.7. Utah Museum of Natural History, Salt Lake City, Utah.
- BAILEY, R.G. (1995). Description of the ecoregions of the United States. Map scale 1:7,500,000. Miscellaneous Publication no. 1391. 2nd ed. USDA Forest Service, Washington DC. Retrieved from <http://www.fs.fed.us/land/ecosysmgmt/index.html>.
- BALDWIN, C.K. (2003). Historical climate analysis. In F.H. Wagner (Ed.), *Rocky Mountain/Great Basin regional climate-change assessment. Report for the U.S. Global Change Research Program* (pp. 58–72). Logan, UT: Utah State University.
- BERTRAND, P.F., ENGLISH, H., URIU, K., & SCHICK, F.J. (1976). Late season water deficits and development of *Cytospora* canker in French prune. *Phytopathology*, 66, 1318–1320.
- BIER, J.E. (1959). The relation of bark moisture to the development of canker diseases caused by native, facultative parasites. I. Cryptodiaportha canker on willow. *Can. J. Bot.*, 37, 229–238.
- BIER, J.E. (1964). The relation of some bark factors to canker susceptibility. *Phytopathology*, 54, 250–253.
- BLOOMBERG, W.J. (1962). *Cytospora* canker of poplars: factors influencing the development of the disease. *Can. J. Bot.*, 40(10), 1271–1280.
- BOND, B.J., & KAVANAGH, K.L. (1999). Stomatal behavior of four woody species in relation to leaf-specific hydraulic conductance and threshold water potential. *Tree Physiol.*, 19, 503–510.
- CHAPELA, I.H. (1989). Fungi in healthy stems and branches of American beech and aspen: a comparative study. *New Phytol.*, 113, 65–75.
- CORE WRITING TEAM, PACHAURI, R.K., & REISINGER, A. (Eds.) (2008). Climate change 2007: synthesis report. Contribution of working groups I, II and III to the fourth assessment report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change, Geneva, Switzerland. Retrieved from http://www.ipcc.ch/publications_and_data/ar4/syr/en/contents.html.
- ESCHENBACH, C., & KAPPEN, L. (1999). Leaf water relations of black alder [*Alnus glutinosa* (L.) Gaertn.] growing at neighboring sites with different water regimes. *Trees*, 14, 28–38.
- FENNEMAN, N.M., WITH COLLABORATION OF JOHNSON, D.W. (1946). Physiographic divisions of the United States. Map, Scale 1:7,000,000. U.S. Department of Interior, Geological Survey, Washington, DC.
- FURLOW, J.J. (1979). The systematics of the American species of *Alnus* (Betulaceae). Part 2. *Rhodora* 81(826), 151–248. Retrieved from <http://www.botanicus.org/item/31753003488290>.
- GHIL, M., ALLEN, R.M., DETTINGER, M.D., IDE, K., KONDRASHOV, D., MANN, M.E., . . . ROBERTSON, A.W. Advanced spectral methods for climatic time series. *Rev. Geophys.*, 40, 1.1–1.41.
- GIORDANO, P.A., & HIBBS, D.E. (1993). Morphological response to competition in red alder: the role of water. *Funct. Ecol.*, 7, 462–468.
- GUYON, J.C., JACOBI, W.R., & MCINTYRE, G.A. (1996). Effects of environmental stress on the development of *Cytospora* canker of aspen. *Plant Dis.*, 80, 1320–1326.
- HACKE, U., & SAUTER, J.J. (1996). Drought-induced xylem dysfunction in petioles, branches, and roots of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn. *Plant Physiol.*, 111, 413–417.
- HACKE, U., STILLER, V., SPERRY, J.S., PITTMANN, J., & MCCULLOH, K.A. (2001). Cavitation fatigue. Embolism and refilling cycles can weaken the cavitation resistance of xylem. *Plant Physiol.*, 125, 779–786.
- HALES, J.E., JR. (1974). Southwestern United States summer monsoon source – Gulf of Mexico or Pacific Ocean? *J. Appl. Meteorol.*, 13, 331–342.
- HANSEN, J., SATO, M., GLASCOE, J., & RUEDY, R. (1998). A common-sense climate index: is climate changing noticeably? *Proc. Natl. Acad. Sci. USA Geophysics*, 95, 4113–4120.
- HINDS, T.E. (1985). Diseases. In N.V. DeByle & R.P. Winokur (Eds.), *Aspen: ecology and management in the western United States. General Technical Report RM-119* (pp. 87–106). Fort Collins, CO: Rocky Mountain Forest and Range Experiment Station, USDA Forest Service. Retrieved from <http://www.treesearch.fs.fed.us/pubs/contents/24942>.

- KEPLEY, J.B., & JACOBI, W.R. (2000). Pathogenicity of *Cytospora* fungi on six hardwood species. *J. Arboric.*, 26, 326–332.
- KITTEL, T.G.F., THORNTON, P.E., ROYLE, J.A., & CHASE, T.N. (2002). Climates of the Rocky Mountains: historical and future patterns. In J.S. Baron (Ed.), *Rocky Mountain futures: an ecological perspective* (pp. 59–82). Covelo, CA: Island Press.
- MCDOWELL, N., POCKMAN, W.T., ALLEN, C.D., BRESHEARS, D.D., COBB, N., KOLB, T., . . . PLAUT, J. (2008). Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol.*, 178, 719–739.
- MCINTYRE, G.A., JACOBI, W.R., & RAMALEY, A.W. (1996). Factors affecting *Cytospora* canker occurrence on aspen. *J. Arboric.*, 22, 229–233.
- RAY, A.J., BARSUGLI, J.J., AVERYT, K.B., WOLTER, K., HOERLING, M., DOESKIN, N., . . . BRADLEY, U. (2008). *Climate change in Colorado: a synthesis to support water resources management and adaptation: a report by the Western Water Assessment for the Colorado Water Conservation Board*. Boulder, CO: University of Colorado at Boulder, Cooperative Institute for Research in Environmental Sciences, Western Water Assessment. Retrieved from http://www.colorado.edu/CO_Climate_Report/index.html.
- RUESS, R.W., MCFARLAND, J.M., TRUMMER, L.M., & ROHRS-RICHEY, J.K. (2009). Disease-mediated declines in N-fixation inputs by *Alnus tenuifolia* to early-successional floodplains in interior and south-central Alaska. *Ecosyst.*, 12, 489–502.
- SALIENDRA, N.Z., SPERRY, J.S., & COMSTOCK, J.P. (1995). Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. *Planta*, 196, 357–366.
- SCHOENEWEISS, D.F. (1975). Predisposition, stress, and plant disease. *Ann. Rev. Phytopathol.*, 13, 193–211.
- SCHOENEWEISS, D.F. (1981). The role of environmental stress in diseases of woody plants. *Plant Dis.*, 65, 308–314.
- SCHOENEWEISS, D.F. (1983). Drought predisposition to *Cytospora* canker in blue spruce. *Plant Dis.*, 67, 383–385.
- SCHRADER, J.A., GARDNER, S.J., & GRAVES, W.R. (2005). Resistance to water stress of *Alnus maritima*: intraspecific variation and comparisons to other alders. *Environ. Exp. Bot.*, 53, 281–298.
- SINCLAIR, W.A., & LYON, H.H. (2005). *Diseases of trees and shrubs* (2nd ed.). Ithaca, NY: Cornell University Press.
- SPERRY, J.S., & SALIENDRA, N.Z. (1994). Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant Cell Environ.*, 17, 1233–1241.
- SPIELMAN, L.J. (1985). A monograph of *Valsa* on hardwoods of North America. *Can. J. Bot.*, 63, 1355–1378.
- STANOSZ, G.R., TRUMMER, L.M., ROHRS-RICHEY, J.K., ADAMS, G.C., & WORRALL, J.J. (2008). Response of *Alnus tenuifolia* to inoculation with *Valsa melanodiscus*. *Phytopathology*, 98 (Suppl.), S150.
- TARDIEU, F., & SIMONNEAU, T. (1998). Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *J. Exp. Bot.*, 49 (Special Issue), 419–432.
- TRUMMER, L. (2004). Alder canker. In D. Wittwer (Ed.), *Forest health conditions in Alaska – 2003. General Technical Report R10-TP-123* (p. 41). Anchorage, AK: USDA Forest Service, Alaska Region, Forest Health Protection. Retrieved from http://forestry.alaska.gov/pdfs/2003_fhp_CONDRPT_web_final.pdf.
- TRUMMER, L. (2006). Alder canker. In C. Snyder (Ed.), *Forest health conditions in Alaska – 2005. Forest Health Protection report R10-PR-5* (p. 52). Anchorage, AK: USDA Forest Service, Alaska Region. Retrieved from http://www.fs.fed.us/r10/spf/fhp/condrpt05/fhp_condrpt_final_2005.pdf.
- UCHYTIL, R.J. (1989). *Betula occidentalis*. In *Fire effects information system* [Online book]. USDA, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. Retrieved from <http://www.fs.fed.us/database/feis/> [accessed November 10 2009].
- WORRALL, J.J. (2009). Dieback and mortality of *Alnus* in the Southern Rocky Mountains, USA. *Plant Dis.*, 93, 293–298.